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# I. Studies on methodical and taxonomical questions

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## Methods of sampling earthworm populations

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### 1. Extraction methods

#### 1.1. Potassium permanganate solution method

Evans and Guild (1947) in their pioneering studies on earthworm populations of agricultural soils in Britain, used potassium permanganate solution to expel worms from the soil, a method limited by the penetration of the solution and the variable response of earthworms to it. The method has been shown (Svendsen 1955) to result for most species in considerable under-estimates of population density and is not recommended.

#### 1.2. Formaldehyde method

The use of formaldehyde as an earthworm expellent was first described by Raw (1959) and has been found particularly useful for studies of *Lumbricus terrestris*. For species with a more horizontal and branching burrow system which the solution cannot easily penetrate it is less suitable and it is also unsuited for studies of aestivating species.

##### 1.2.1. *Concentration of solution*

At Rothamsted, Raw tested solution strengths of 25 ml of 40 per cent formalin in 1 l gallon of water, approximately a 0.55 per cent solution, and a quarter of this concentration, approximately 0.14 per cent. The less concentrated solution expelled less than 50 per cent of the number of worms expelled by the stronger solution in a population comprising mainly *Allolobophora chlorotica*, *A. caliginosa* and *A. rosea*. Subsequently concentrations of 0.165 per cent, 0.275 per cent and 0.55 per cent were compared at Merlewood and were found equally effective in expelling both *Allolobophora* and *Lumbricus* species.

The procedure used at Merlewood is to apply three lots of 2 gallons (9 litres) of solution at 10 minute intervals to each quadrat of 0.5 m². In tests in which four more lots of solution were applied, 95 per cent of the final catch had been taken after the first three applications.

##### 1.2.2. *Correltion for soil temperature and moisture*

Both soil temperature and the soil moisture content have been found to have an effect on the number of worms expelled by formaldehyde solution (Satchell 1967). In studies on *L. terrestris* in English woodland the maximum response to formaldehyde occurred

at about 10.6 °C and the numbers expelled increased linearly with soil moisture content to 38 % and decreased at 44 %. The correction term found appropriate (LAKHANI and SATCHELL unpublished) was:

Estimated population =

Observed population × exponential [0.0075 (T-10.6²)] × exponial [— 0.0214 (M-40)]

where T = soil temperature at 10 cm in °C

and M = % soil moisture content.

The model on which this formula is based is discussed by SKELLAM (1967). It should be stressed that the values of the constants in the correction term apply only to *Lumbricus terrestris* on the sites studied.

### 1.2.3. *Efficiency of the method*

RAW (1959) compared a formalin treatment using 0.55 % formaldehyde solution at the rate of approximately 24 *l*/m² (2 gallons per 4 sq. ft.) with hand sorting to a depth of 20 cm. (8 in.). The latter method produced fewer *L. terrestris* than the formalin treatment so the comparison throws no light on the efficiency of formalin as an expellent for this species. In grassland, hand sorting yielded almost two and a half times as many specimens of other species as were expelled by formaldehyde. Preliminary digging and hand sorting tests at Merlewood indicate that the formalin method can be used to obtain satisfactory estimates of *Lumbricus terrestris* populations provided the appropriate correction is made for departures from optimum soil temperature and moisture content.

## 1.3. Hand sorting

Hand sorting, though laborious, is widely used as a sampling method and for assessing the efficiency of other methods. Its efficiency has been tested by RAW (1960) and NELSON and SATCHELL (1962).

RAW carried out tests on a wet hill grassland soil with a thick mat of vegetation in which worms were confined to the top of the profile. Samples were sorted by hand and the vegetation and mineral soil were then re-examined separately. The vegetation was teased out in water to which a few drops of formalin had been added to make the worms wriggle. The mineral fraction was worked through a 2 mm sieve to break up soil crumbs and was then immersed in magnesium sulphate solution on which the worms floated. Of the total worms found of all species, 52 % of the number and 84 % by weight were found by hand sorting. Only 38 % of the total number and 57 % of the total weight of a small species, *Eiseniella tetraedra*, were found by hand sorting. From similar tests on a heavy arable soil, 59 % of the total number and 90 % of the total weight were found by the hand method.

NELSON and SATCHELL tested the method by sorting prepared samples into which known numbers of worms had been introduced. Samples of rough grassland were freed of live worms by freezing and specimens of various sizes and species, including pigmented and unpigmented forms, were introduced. Of 924 worms introduced, 93 % (99 % by weight) were found by hand sorting but only 80 % of immature pigmented *Allolobophora chlorotica* and 74 % of *Lumbricus castaneus* were recovered. In general the method seemed to give a satisfactory recovery of individuals weighing more than 0.2 g live weight but not of smaller worms. For studies aimed at producing life tables, hand sorting would seem unlikely therefore to give a satisfactory recovery of the lowest age classes.

These tests were carried out with the operators knowing that their efficiency was being checked and may therefore have given an above average recovery.

### 1.4. Wet sieving

Washing soil with a jet of water through a series of sieves in the manner of MORRIS (1922) has been used for collecting earthworms in studies concerned also with other faunal groups. There appears to be no published study of the efficiency of the method for extracting earthworms but its various disadvantages e. g. damage to specimens and the long time and labour required to separate them from the residues, are listed by LADELL (1936). RAW (1960) found hand sorting much quicker than washing for both light and heavy soils.

### 1.5. Flotation

The method used by RAW (1960) for extracting small species from soils unsuitable for hand sorting was based on an apparatus described by SALT and HOLLICK (1944). It has the advantage that it can be adapted to extract earthworm egg capsules and so enable all stages of the population to be estimated. A large flotation apparatus is currently under development by Mr B. HANSON at University College, London (O'CONNOR, at press).

### 1.6. Heat extraction

This method which operates on the principle of the BAERMANN funnel (BAERMANN 1917) has been used for extracting small surface living species from matted turf which would be difficult to sort by hand. The apparatus consisted of a plastic baby-bath, 55 × 45 cm, in which rested a wire gauze sieve 5 cm from the bottom of the bath. A battery of fourteen 60 watt light bulbs held in a hardboard sheet rested on the rim of the bath on two battens so that when a soil sample was placed in the sieve the light bulbs were suspended about 2 cm above it. The bath was filled with water to about half way up the soil sample which was heated by the bulbs for three hours. The heating unit, sieve and contents were then removed, and the worms were collected from the water immediately. The soil samples used in this apparatus were generally 20 × 20 cm × 10 cm deep.

The method has been found more effective than either formalin extraction or hand sorting for a population of *Bimastos eiseni* in a dense mat of *Deschampsia flexuosa*.

### 1.7. Trapping

Trapping methods are unlikely to yield accurate population estimates but can form a useful means of studying activity patterns (SVENDSEN 1957) where population densities are low.

## 2. Determination of size and number of samples

### 2.1. Sampling unit area

A number of workers have followed WILCKE (1955) in selecting a sample unit area of 50 × 50 cm and a depth of 40 or 50 cm. ZICSI (1958) compared samples of this size with units of 25 × 25, 50 × 100 and 100 × 100 cm. He found that the number of worms per m² recovered by hand sorting decreased with increasing size of sample, indicating an increase in tediousness and hence of inefficiency in sorting the larger samples. In an arable soil, the average number of worms estimated per m² from 50 units of each size were 25 × 25 84.5; 50 × 50 77.7; 50 × 100 62.8; 100 × 100 62. A tool for taking soil samples of 25 × 25 cm (1/16 m²) to a depth of 20 cm is described by ZICSI for use in soils which are not too stony. SVENDSEN (1955) used a steel scoop of semicircular crossection to cut cores 283 sq. cm (1/35 m²) in area and 20 cm deep. The minimum sample area which will prove satisfactory will depend largely on the density of the population to be sampled.

### 2.2. Number of sampling units

The number of samples which needs to be taken to ensure a population estimate of specified accuracy will depend on their size and on the density and degree of aggre-

gation of the population. This must be ascertained in the particular case by trial sampling, but the following data from two typical temperate European sites (Table 1) indicate the sampling variance likely to be encountered.

It will be found that a sampling error of less than 10% of the mean cannot generally be obtained without an excessive sampling effort.

Table 1 Numbers of samples required for estimating population means of given sampling error

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *L. terrestris* in ten 0.5 m² quadrats | | | | | | | | | |
| 1. A Merlewood population sampled by the formalin method | 25 | 28 | 25 | 35 | 15 | 24 | 15 | 18 | 17 | 22 |
| | *A. caliginosa*/sq. ft. | | | | | | | | | |
| 2. A Rothamsted population sampled by an electrical method | 7.7 | 1.0 | 5.4 | 6.0 | 4.2 | 7.0 | 3.8 | 11.8 | 3.7 | 7.0 |

Number of samples required to give a sampling error of the mean equal to

| | 5% | 10% | 20% of the mean |
|---|---|---|---|
| Merlewood population | 32 | 8 | 2 |
| Rothamsted population | 103 | 26 | 7 |

## 3. Biomass Estimation

### 3.1. Loss in weight during preservation

The British Museum recommends (anon 1932) preservation of earthworms in 70—90% alcohol after a preliminary fixing in 10% formaldehyde. However most ecologists, as distinct from taxonomists, followed EVANS and GUILD (1947) in using formalin as a preservative at concentrations of 4—10%. At Merlewood, *L. terrestris* preserved in 70% alcohol were found to have lost 10% of their fresh weight in four days and RAW (1962) stated that the weight of worms preserved in 5% formalin is about 25% less than the fresh weight. Fifty specimens of *L. terrestris* weighed 10% less than their fresh weight after 7 days and there is some evidence that greater weight losses may occur in other species. This factor must therefore be taken into account when biomass estimates are to be based on samples of preserved worms.

### 3.2. Conversion of fresh weight to dry weight

The analysis of the size class distribution of an earthworm population requires large numbers of worms to be weighed individually. If they are first collected into preservative this must be removed from the surface of the worm before weighing. The common practice of rolling the specimen on a cloth or filter paper is difficult to standardize and can be a source of serious error. It can be eliminated by determining the live weight/dry weight ratio and applying this to individual worm weights obtained from subsequent samples which have been collected into preservative, oven dried at 105 °C and weighed from a desiccator. In studies where a life table can be determined solely from samples collected from the field it may be possible to work entirely on a dry weight basis but where the growth rate has to be computed in part from culture data the use of live weights is unavoidable. Regression analysis of the data from 44 specimens of *L. terrestris* which were weighed live, preserved for 4 days in 4% formalin, dried at 105 °C and re weighed gave the relation

$$1 \text{ g dry weight} = 5.5 \text{ g live weight.}$$

In preparing estimates of the biomass of earthworm populations the weight of the gut contents should not be overlooked. A sample of 10 *L. terrestris* taken from the field and dissected had gut contents weighing 20% of the fresh weight of the whole worm but this value varied with the size of the worm. In a further sample of 30 worms selected

to include a wide size range, the dry weight of the gut contents was on average 29 % of the dry weight of the whole worm and ranged from 26 % in worms of about 0.5 g live weight to 41 % in worms of 7 g live weight. The regression of percentage weight of gut content on body size was significant at the 1 % probability level. This result may have arisen from loss of gut material during dissection of the smaller worms or it may indicate that the gut is larger in proportion to the body in larger worms or that the larger worms ingest a higher proportion of mineral matter in their diet than the smaller worms. BOUCHE (1966a) has described a method of washing out the gut contents of large lumbricids employing a catheter and syringe and (BOUCHÉ 1966b) a technique for measuring biovolume as an alternative to biomass.

## 4. Estimation of relative productivity

Relative productivity is defined as the ratio of the annual turnover of biomass to the average prevailing biomass. A first attempt to estimate it for two populations of *L. terrestris* will be described in a forthcoming publication by LAKHANI and SATCHELL.

The estimates are based on the size class structure of the populations, their growth rates and their mortality and survivorship curves. The average biomass of the populations was about 145 g/m² and the estimates suggest an annual production of about 65 g/m².

## 5. Literature


Anon., 1932. Instructions for collectors No. 2 Worms. British Museum, Natural History, London.

BAERMANN, G., 1917. Eine einfache Methode zur Auffindung von Ankylostomum-(Nematoden)-Larven in Erdproben. Meded. geneesk. Lab. Weltevr. 41—7.

BOUCHE, M. B., 1966a. Sur un nouveau procédé d'obtentian de la vacuité artificielle du tube digestif des Lombricides. Rev. Ecol. Biol. Sol. T. **III**. 3, 479—482.

— 1966b. Application de la volumétrie à l'évaluation quantitative de la faune endogée. Rev. Ecol. Biol. Sol. T. **III**. 1, 19—30.

EVANS, A. C., and W. J. MCL. GUILD, 1947. Studies on the relationship between earthworms and soil fertility. I. Biological studies in the field. Ann. appl. Biol. **34**, 307—330.

LADELL, W. R. S., 1936. A new apparatus for separating insects and other arthropods from the soil. Ann. appl. Biol. **4**, 862—879.

LAKHANI, K. H., and J. E. SATCHELL (in prep.).

MORRIS, H. M., 1922. On a method of separating insects and other arthropods from the soil. Bull. ent. Res. **13**, 197.

NELSON, J. M., and J. E. SATCHELL, 1962. The extraction of Lumbricidae from soil with special reference to the hand sorting method. Pp. 294—299 in "Progress in Soil Zoology". Ed. P. W. MURPHY. Butterworths, London.

O'CONNOR, F. B. (at press). The estimation of earthworm populations with a note on a mechanical method of extraction. Proc. IBP technical meeting on Methods of Study in Soil Zoology. UNESCO, Paris.

RAW, F., 1959. Estimating earthworm populations by using formalin. Nature, London **184**, 1661—1662.

— 1960. Earthworm population studies: a comparison of sampling methods. Nature, Lond. **187**, 257.

— 1962. Studies of earthworm populations in orchards. I. Leaf burial in apple orchards. Ann. appl. Biol. **50**, 389—404.

SALT, G., and F. S. J. HOLLICK, 1944. Studies of wireworm populations. I. A census of wireworms in pasture. Ann. appl. Biol. **31**, 52—64.

SATCHELL, J. E., 1967. Lumbricidae. In: A. BURGES and F. RAW (ed.): Soil Biology, Academic Press, London and New York, pp. 259—322.

SKELLAM, J. G., 1967. Productive processes in animal populations considered from the biometrical standpoint. In: PETRUSEWICZ, K. (ed.): Secondary Productivity of Terrestrial Ecosystems. Proc. IBP. Symposium, Warsaw.

SVENDSEN, J. A., 1955. Earthworm population studies: a comparison of sampling methods. Nature, Lond. **175**, 864.

— 1957. The behaviour of lumbricids under moorland conditions. J. Anim. Ecol. **26**, 423—39.

Wilcke, D. E., 1955. Kritische Bemerkungen und Vorschläge zur quantitativen Analyse des Regenwurmbesatzes bei zoologischen Bodenuntersuchungen. Z. Pflanzenernähr. Bodenkunde **68**, 44—49.

Zicsi, A., 1958. Determination of number and size of sampling unit for estimating lumbricid populations of arable soils. In: Murphy, P. W. (ed.): Progress in Soil Zoology. Butterworths. London. pp. 68—71.

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